

The Population Structure of *Phytophthora infestans* from the Toluca Valley of Central Mexico Suggests Genetic Differentiation Between Populations from Cultivated Potato and Wild *Solanum* spp.

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ABSTRACT

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The population structure of *Phytophthora infestans* in the Toluca Valley of central Mexico was assessed using 170 isolates collected from cultivated potatoes and the native wild *Solanum* spp., *S. demissum* and *S. xedinense*. All isolates were analyzed for mitochondrial DNA (mtDNA) haplotype and amplified fragment length polymorphism (AFLP) multi-locus fingerprint genotype. Isolate samples were monomorphic for mtDNA haplotype because all isolates tested were of the 1a haplotype. A total of 158 multilocus AFLP genotypes were identified among the 170 *P. infestans* isolates included in this study. *P. infestans* populations sampled in the Toluca Valley in 1997 were highly variable and almost every single isolate represented a unique genotype based on the analysis of 165 AFLP marker loci. Populations of *P. infestans* collected from the commercial potato-growing region in the valley, the subsistence potato production

area along the slopes of the Nevado de Toluca, and the native *Solanum* spp. on the forested slopes of the volcano showed a high degree of genetic diversity. The number of polymorphic loci varied from 20.0 to 62.4% for isolates collected from the field station and wild *Solanum* spp. On average, 81.8% (135) of the AFLP loci were polymorphic. Heterozygosity varied between 7.7 and 19.4%. Significant differentiation was found at the population level between strains originating from cultivated potatoes and wild *Solanum* spp. ($P = 0.001$ to 0.022). Private alleles were observed in individual isolates collected from all three populations, with numbers of unique dominant alleles varying from 9 to 16 for isolates collected from commercial potato crops and native *Solanum* spp., respectively. Four AFLP markers were exclusively found present in isolates collected from *S. demissum*. Indirect estimation of gene flow between populations indicated restricted gene flow between both *P. infestans* populations from cultivated potatoes and wild *Solanum* hosts. There was no evidence found for the presence of substructuring at the subpopulation (field) level. We hypothesize that population differentiation and genetic isolation of *P. infestans* in the Toluca Valley is driven by host-specific factors (i.e., R-genes) widely distributed in wild *Solanum* spp. and random genetic drift.

The central highlands of Mexico, which include the Toluca Valley, are considered the center of diversity of the oomycete *Phytophthora infestans* (Mont.) de Bary, causing potato late blight (13,14,20,37). Genetic diversity of virulence (31,34,39,46,48), allozymes (20,45), and restriction fragment length polymorphism (RFLP) loci (20) has been extensively studied using the *P. infestans* population in the Toluca Valley. Both mating types can be found in approximately a 1:1 ratio (20,22) and oospores are commonly found in potato crops (16,33) and native *Solanum* spp. (12). The population of *P. infestans* in the Toluca Valley of central Mexico is therefore believed to be the most diverse in the world (14,17,20,45). The Toluca Valley is located at an altitude of 2,600 m and mountain ridges surround the valley itself (22). On the southwest, the valley is delimited by the extinct volcano Nevado de Toluca, reaching an altitude of 4,690 m. Potatoes are grown up to approximately 3,500 m altitude and wild species of *Solanum* occur at the edges of pine and fir forests up to about 3,800 m altitude (38–42).

The meta-population structure of *P. infestans* in the Toluca Valley can be differentiated into three populations based on host-plant and potato production systems. In the central valley, high-input potato fields are prevalent, whereas low-input potato production is found on the lower slopes of the volcano. The third population refers to *P. infestans* infecting several wild *Solanum* spp. native to central Mexico (41) (Fig. 1).

Reports of specialized strains with differential pathogenicity to potato and tomato fuelled speculation about the presence of host preference and specialization within *P. infestans* (26,47). Results from recent work on late blight in Ecuador are suggesting that population differentiation and host specificity in *P. infestans* can be found. Ordoñez et al. (35) reported on an Ecuadorian *Phytophthora* A2 population (closely resembling *P. infestans*) from a native *Solanum* spp. This A2 population appears to be strictly isolated from potato strains by host-plant specificity.

Although host-plant specialization has been reported for these systems, limited information is available on presence and implications of population differentiation and isolating mechanisms in *P. infestans* from the Toluca Valley. Earlier reports (14,22,23,29) used metalaxyl resistance and neutral *peptidase* (*Pep*) and *glucose-6-phosphate isomerase* (*Gpi*) allozyme markers to explore the possibility of population differentiation of *P. infestans* in the

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Toluca Valley. Metalaxyl resistant strains were found on commercial potato crops (heavily treated with metalaxyl) and native *Solanum* spp. (not treated with metalaxyl) in similar frequencies, suggesting that strains migrate freely between potato crops and native *Solanum* spp. Allozyme data indicated that heterogeneity and genetic diversity in *P. infestans* populations declined from valley to rural and wild *Solanum* spp. Little support was found for the hypothesis that the native hosts *S. demissum* and *S. xedinense* act as differential host plant species harboring specialized populations of *P. infestans*. Some indications for the presence of isolating mechanisms in *P. infestans* populations from the Toluca Valley have been reported (12). Significant differences in oospore production were observed between in vitro crosses of *P. infestans* strains collected from potato and *S. demissum*. Isolates originating from the native host *S. demissum* produced significantly more oospores in crosses with compatible strains collected from the same host species compared with crosses with isolates collected from cultivated potatoes.

Our previous studies provided only few indications for the presence of host-specific populations of *P. infestans* in the Toluca Valley (12,23). Neutral DNA markers had until now not been deployed to measure genetic diversity and gene flow between host-specific populations of *P. infestans* to determine whether population differentiation is likely to occur in the center of diversity of the late blight pathogen. The objectives of this study were to (i) characterize the genetic structure of *P. infestans* populations in the Toluca Valley using mitochondrial DNA (mtDNA) haplotypes and amplified fragment length polymorphism (AFLP) markers to determine genetic diversity and gene flow of *P. infestans* at its center of diversity; (ii) compare the relative performance of AFLP and RG57 RFLP genomic fingerprinting in a sexual reproducing late

blight population; and (iii) determine whether populations of *P. infestans* on native *Solanum* spp. are merely derived from populations on cultivated potatoes or whether they should be regarded as host-specific subpopulations of the total valley population.

MATERIALS AND METHODS

Definitions. An operational definition of population was used as described in Grünwald et al. (22). Populations of *P. infestans* were defined by both host composition and potato growing practice in regions in which they were sampled: (i) large potato fields in the central valley where late blight susceptible cvs. Alpha and Atlantic are grown (VALLEY), (ii) small fields containing cultivar mixtures of locally grown potato cultivars locally known as “papas criollas” or commercial cultivars (mostly cvs. Alpha and Rosita) grown by subsistence farmers on the slopes of the volcano (RURAL), and (iii) patches of *S. demissum* (200 to 5,000 plants per population) and *S. xedinense* (usually individual plants within “Criolla fields”) (WSS). Individual sampling sites (fields or patches of wild *Solanum* spp.) sampled within one of the three defined populations were considered subpopulations of assigned populations.

Isolates. A total of 371 isolates were collected from the populations WSS, VALLEY, and RURAL in 1997 (Table 1). Collections were made in three areas in the Toluca Valley using a hierarchical sampling strategy described previously (22). The sampling scheme consisted of isolating 10 to 15 isolates each from at least 10 fields in the valley (VALLEY), on the slopes of the volcano (RURAL), and 10 patches of wild *Solanum* spp. (WSS). Each isolate originated from a randomly selected single leaf lesion of *S. tuberosum*, *S. demissum*, or *S. xedinense*. These populations were charac-

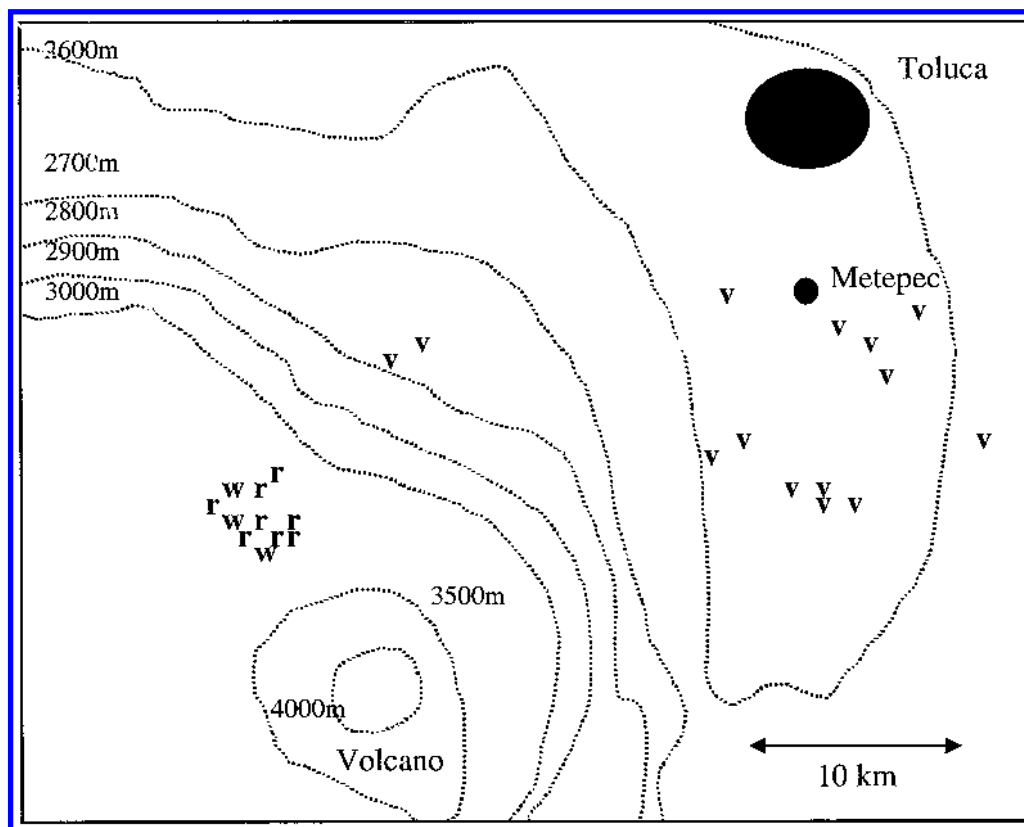


Fig. 1. Sites of collection of *Phytophthora infestans* isolates from the Toluca Valley located in the state of Mexico. Isolates were obtained in 1997 and 1998 from three different pathogen-plant habitats including (i) populations of *P. infestans* infecting wild solanaceous host plants growing on the slopes of the volcano Nevado de Toluca (3,000 to 3,500 m above sea level) where no fungicides are applied and all plants are expected to carry R-genes (w = WSS); (ii) on potatoes in rural low-input potato fields on the slopes of the volcano (3,000 to 3,500 m above sea level) where fungicides are applied sporadically and R-genes occur (r = RURAL); and (iii) on potatoes in high-input agriculture where fungicides are applied every 2 to 3 days and only susceptible cultivars are grown (v = VALLEY).

terized previously using mating type, isozyme, metalaxyl, and RFLP markers (22,23). We used 170 isolates that were available in the *P. infestans* isolate collection at Plant Research International (Wageningen, the Netherlands) and assumed they represent a random subsample of the isolates collected during multiple field collections in 1997. Isolation and culture were as described previously (8,12,22).

DNA extraction. Isolates were grown for 10 to 14 days at 20°C in pea broth (with 200 mg liter⁻¹ of ampicillin added) prepared by autoclaving 120 g of frozen peas in 1 liter of water. The peas were removed by filtering through cheesecloth and the broth was autoclaved again. The mycelium was grown on pea broth, harvested, lyophilized, and stored at -80°C. Lyophilized mycelium (10 to 20 mg) was ground in microcentrifuge tubes with a pestle and sterile sand. Total DNA was extracted with the Puregene kit (Gentra/Biozym, Landgraaf, the Netherlands) according to manufacturer's instructions. DNA was dissolved in 100 µl of TE (10 mM Tris-HCl [pH 8.0] and 1 mM EDTA [pH 8.0]) and stored at -20°C.

Mitochondrial haplotypes. The P1 (1,118 bp), P2 (1,070 bp), P3 (1,308 bp), and P4 (964 bp) regions of the mitochondrial genome were amplified using primers and methods described by Griffith and Shaw (21). Reactions were performed in a thermocycler (PTC200; MJ Research/Biozym, Landgraaf, The Netherlands). Polymerase chain reaction (PCR) products, digested with restriction enzymes *Cfo*I, *Msp*I, and *Eco*RI yields restriction fragment patterns that can be classified into four different mtDNA haplotypes: Ia, Ib, IIa, and IIb (21).

RG57 RFLP fingerprinting. Moderately repetitive clone RG57 (18) was used for Southern blot analysis using the Renaissance nonradioactive detection kit as described by the manufacturer (New England Nuclear, Boston, MA).

Fluorescent AFLP. DNA (250 ng) was digested in a 50-µl reaction volume with *Eco*RI (10 units) and *Mse*I (10 units) for 6 h

at 37°C in restriction ligation buffer (10 mM Tris/Ac [pH 7.5], 10 mM MgAc, 50 mM KAc, 5 mM dithiothreitol, and 50 ng µl⁻¹ of bovine serum albumin). Digestion was confirmed on agarose gels. Restriction fragments were ligated to *Mse*I adapters (5'-GACGATGAGTCCTGAG and TACTCAGGACTCAT-5') and *Eco*RI adapters (5'-CTCGTAGACTGCGTACC and CTGACGC-ATGGTTAA-5') using 0.1 µM *Eco*RI adapter, 1.0 µM *Mse*I adapter, 0.2 mM ATP, and 2.4 Weiss-U T4 DNA ligase (Amersham Pharmacia Biotech, Uppsala, Sweden) (1). Ligation was performed overnight at 10 to 12°C and the ligation products were diluted 10 times with filtered ultra pure water. Nonselective PCR amplification was performed using primers *Eco*00 (5'-GACT-GCGTACCAATTC) and *Mse*00 (5'-GATGAGTCCTGAGTAA). Nonselective PCR amplifications were performed in a thermocycler (PTC200; MJ Research/Biozym) as described previously (5). The amplified restriction fragment products were checked on 1.0% agarose gels. Selective PCR was performed in a 20-µl reaction volume with 5 µl of 20× diluted amplification products as described previously (1), but with 200 µM dNTP and 5 ng of Cy5-labeled fluorescent *Eco*21 primer (5'-GACTGCGTACCAATTCG-G) and 30 ng of *Mse*16 (5'-GATGAGTCCTGAGTAACC) primer.

Products were loaded on Sequagel (Biozym) polyacrylamide gels and run on an ALFexpress automatic sequencer (Pharmacia). Conditions were 1,500 V, 60 mA, 35 W, and 55°C. On each gel, 36 samples were loaded together with flanking Cy5-labeled fluorescent 50-bp ladders (Amersham) and two reference isolates (PIC99016 and NL-VK6C).

Data analysis. Each isolate was classified into an mtDNA haplotype after visualization of the restriction fragments on agarose gels using ethidium bromide under UV illumination. AFLP patterns were analyzed using Imagemaster ID software (Pharmacia Biotech), manually correcting for faint bands and exclusion of controversial bands. A total of 158 distinct and reproducible AFLP bands were identified using primers *Eco*21 and *Mse*16. Bands

TABLE 1. Sample sizes and indicators of genotypic diversity for three Mexican *Phytophthora infestans* field populations sampled in the Toluca Valley in 1997 based on amplified fragment length polymorphism (AFLP) fingerprinting^a

Population	Location	<i>n</i> ^b	<i>g</i> ^c	<i>h</i> ₀ ^d	<i>P</i> ^e	<i>H</i> ^f	Metalaxyl tolerance ^g		
							Sensitive	Intermediate	Resistant
VALLEY	Field station	12	12	2.485	20.0	0.077	10	1	1
	Commercial fields	41	41	3.714	44.2	0.153	28	7	6
RURAL	Low-input cultivation	35	34	3.545	38.2	0.192	27	5	3
WSS	Wild <i>Solanum</i> spp.	80	75	4.270	62.4	0.194	71	9	2
Overall		170	158	5.023	81.8	0.210	136	22	12

^a A total of 165 marker loci were obtained.

^b *n* = population size.

^c *g* = number of multilocus AFLP genotypes in population.

^d *h*₀ = Shannon index.

^e *p* = percentage of polymorphic loci (99% criterion).

^f *H* = average unbiased proportion heterozygosity.

^g Data from Grünwald et al. (22).

TABLE 2. Sample sizes and indicators of genotype diversity for four Mexican subpopulations of *Phytophthora infestans* collected on wild *Solanum* host plant species in the valley of Toluca in 1997 based on amplified fragment length polymorphism fingerprinting^a

Subpopulation	Host species	<i>n</i> ^b	<i>g</i> ^c	<i>h</i> ₀ ^d	<i>P</i> ^e	<i>H</i> ^f	Metalaxyl tolerance		
							Sensitive	Intermediate	Resistant
Patch 1	<i>S. demissum</i>	23	21	3.015	35.2	0.122	22	1	0
Patch 3	<i>S. demissum</i>	19	18	2.871	37.6	0.143	17	1	1
Patch 3	<i>S. xedinese</i>	13	12	2.458	27.3	0.111	9	3	1
Patch 4	<i>S. demissum</i>	18	17	2.813	30.9	0.114	18	0	0
Overall		73	70	4.234	61.4	0.190	66	5	2

^a A total of 165 marker loci were obtained.

^b *n* = population size.

^c *g* = number of genotypes in population.

^d *h*₀ = Shannon index.

^e *p* = percentage of polymorphic loci (99% criterion).

^f *H* = average unbiased proportion heterozygosity.

were treated as putative single AFLP loci and a binary matrix containing the presence or absence of these reproducible bands was constructed and used for further analysis. The matrix is available from W. G. Flier upon request. Statistical analyses were conducted with POPGENE 1.31, available at no cost from the University of Alberta, Canada, and TFPGA (Tools for Population Genetic Analyses, version 1.3), available at no cost from M. P. Miller at Northern Arizona University, Flagstaff. Each AFLP band was assumed to represent the dominant genotype at a single locus while the absence of that same band represents the alternate homozygous recessive genotype. We assumed a diploid model with two alleles per locus and estimated the frequency of the recessive allele by a Taylor expansion estimator (27), which proves an alternative and apparently less biased estimator compared with the square root of the frequency of "blanks" seen at a particular AFLP locus. Genotypic diversity analysis was used to determine the distribution of genetic diversity among populations (VALLEY, RURAL, and WSS) and among subpopulations of *P. infestans* collected from patches of *S. demissum* or *S. xedinense*. Heterozygosity and percent polymorphic loci (99% criterion) were estimated for populations and WSS subpopulations. Genotypic diversity was calculated using Shannon's information index (42). Pair-wise measures of Rogers' modified genetic distance and population differentiation using Nei's coefficient of differentiation (G_{ST}) (32,44) was calculated using POPGENE. Population structure was analyzed using F statistics (49,50) in order to test the significance of the different statistics for the null hypothesis of no differentiation at the corresponding hierarchical level. Permutation and resampling tests (jackknifing and bootstrapping) were carried out to calculate estimates for standard errors. In our analysis, we used a two-level hierarchy with corresponding statistics F_{pop} and F_{site} for sites within populations and isolates within sites, respectively. Populations were defined according to their geographic location and potato production system. Differentiation among populations was estimated using an exact test (36) and by indirect estimation of gene flow using G_{ST} with $Nm = 1/4(1 - G_{ST})/G_{ST}$ (32,43), where N is the effective population size, m is the immigration rate, and Nm is the average number of migrants among populations per generation (43). Cluster analysis of multilocus AFLP genotypes was based on allele frequencies observed for each population. A phenogram was constructed using the unweighted pair-group method of averages (UPGMA) algorithm from a Rogers' modified genetic distance matrix (50). Bootstrap sampling (1,000 replicates) was performed for parsimony analysis of the constructed phenogram (11).

RESULTS

mtDNA haplotypes. All 170 *P. infestans* isolates sampled from the three subpopulations in the Toluca Valley were of the Ia haplotype.

AFLP fingerprinting. A total of 158 distinct multilocus AFLP genotypes were found among the 170 *P. infestans* isolates included in this study (Table 1). In general, *P. infestans* populations

sampled in the Toluca Valley in 1997 were highly variable, and almost every single isolate represented a unique genotype, based on the analysis of 165 AFLP marker loci. Populations of *P. infestans* collected from the commercial potato-growing region in the valley, the subsistence potato production area along the slopes of the Nevado de Toluca, and from wild *Solanum* spp. on the forested slopes of the volcano showed a high degree of genetic variability. The number of polymorphic loci (based on the 99% criterion) varied from 20.0 to 62.4% for isolates collected from the field station and wild *Solanum* spp., respectively (Table 1). Overall, 81.8% (135) of the AFLP loci were polymorphic. Heterozygosity obtained by indirect estimates of allele frequencies based on a Taylor expansion (27) varied between 7.7 and 19.4% (Table 1). Again, isolates collected from wild *Solanum* hosts showed the highest level of heterozygosity, whereas isolates from the experimental plots at the field station showed only half of the variation compared with isolates from commercial fields and locally grown potato cultivars. Genotypic diversity measurements between field populations revealed a similar pattern with the highest genotype diversity for the isolates collected on native *Solanum* spp. (Table 1). Most parameters describing the genetic variation at the subpopulation level were very similar, based on the results of the fine-grain sampling of *P. infestans* isolates from five patches of native *Solanum* spp. (22), mainly consisting of *S. demissum* and, in the case of patch 3, also including isolates collected from *S. xedinense* (Table 2). Sample sizes varied between 2 and 32 isolates per patch for patch 9 and 3, respectively. Heterozygosity and the level of genotypic diversity were comparable between four distinguished subpopulations of *P. infestans* collected from *S. demissum* and *S. xedinense* with sample sizes exceeding 10 isolates (Table 2). The number of polymorphic loci varied between subpopulations (Table 2), but is clearly associated with the number of unique genotypes in a sample ($r = 0.961$).

Metalaxyl sensitivity. Metalaxyl resistant or intermediate tolerant isolates were observed for all three *P. infestans* populations sampled (22,23) (Table 1). Metalaxyl insensitivity was found in isolates originating from wild *Solanum* spp. Resistance to metalaxyl was detected in isolates from all patches, except patch 4, with relatively high numbers of intermediate or resistant isolates originating from *S. xedinense* (Table 2). A total of 4 isolates out of 13 collected from *S. xedinense* were resistant or intermediate, whereas 3 of 60 isolates from *S. demissum* were determined to be less sensitive to metalaxyl (Table 2). Pearson's χ^2 test

TABLE 4. Matrix of Rogers' modified genetic distance coefficients for three subpopulations of *Phytophthora infestans* sampled in the Toluca Valley^a

Population	VALLEY	RURAL	WSS
VALLEY	...	0.176	0.204
RURAL	0.159	...	0.185
WSS	0.218	0.244	...

^a Below the diagonal, genetic distances based on 165 amplified fragment length polymorphism loci; above the diagonal, genetic distances based on 26 RG57 restriction fragment length polymorphism loci.

TABLE 3. Sample sizes and indicators of genotype diversity for three subpopulations of *Phytophthora infestans* collected in the valley of Toluca in 1997 based on either amplified fragment length polymorphism (AFLP) fingerprinting or RG57 restriction fragment length polymorphism (RFLP) fingerprinting

Subpopulation	n^a	g^b		h_0^c		P^d		H^e	
		AFLP	RFLP	AFLP	RFLP	AFLP	RFLP	AFLP	RFLP
VALLEY	14	14	13	2.639	2.540	32.73	50.00	0.13	0.19
RURAL	19	14	18	2.944	2.871	30.91	61.54	0.12	0.18
WSS	7	6	6	1.513	1.513	21.81	34.62	0.10	0.15

^a n = population size.

^b g = number of genotypes in population.

^c h_0 = Shannon index.

^d P = percentage of polymorphic loci (99% criterion).

^e H = average unbiased proportion heterozygosity.

revealed a positive association between host plant species and metalaxyl insensitivity ($P = 0.05$).

Comparison between AFLP and RFLP. AFLP and RG57 RFLP fingerprinting applied to a subsample of 40 *P. infestans* isolates revealed comparable levels of genetic variation at the DNA level (Table 3). A fourfold increase in the number of informative loci was observed when AFLP fingerprinting was used to characterize the 40 isolates, but this did not lead to significant changes in indices used to describe genotype diversity (Table 3). The calculated Rogers' modified genetic distances between VALLEY, RURAL, and WSS subpopulations based on either AFLP or RFLP data were very similar (Table 4), although a slightly greater genetic distance between WSS and VALLEY/RURAL was detected with AFLP data (Table 4; Fig. 2). A higher proportion of similar bootstrapping replicates was detected in UPGMA cluster analysis when AFLP data were used (0.524 to 0.861 for RFLP and AFLP, respectively).

Population differentiation. Results based on the test for population differentiation indicated strong population substructuring between *P. infestans* populations originating from cultivated potato and wild *Solanum* spp. (Table 5). The χ^2 test statistic probabilities for the pair-wise comparisons between VALLEY, RURAL, and WSS populations lead to rejection of the null hypothesis stating an absence of population differentiation in the case of VALLEY versus WSS ($P = 0.001$) and RURAL versus WSS ($P = 0.022$). Genotypic diversity measures were similar for 28 pairs of subpopulations of *P. infestans* in the Toluca Valley (Table 6). Genetic identity, as presented by Nei's genetic identity index, revealed only limited differences between the populations included in this study. Based on Rogers' genetic distance coefficients, VALLEY and RURAL populations are more closely related to each other than to subpopulations of *P. infestans* collected from wild *Solanum* spp. (WSS population) (Fig. 3). Rare alleles were observed in individual isolates collected from all three populations, with the number of unique dominant alleles varying from

9 to 16 for VALLEY and WSS, respectively. Four AFLP markers were exclusively found in isolates collected from *S. demissum* (varying from 7 to 11 unique genotypes per marker). Sampling effects are not likely to explain the observed differences in frequency of the four AFLP alleles (Chi-square based on goodness of fit test; data not shown). Indirect estimation of gene flow between populations using N_m as a measurement of population differentiation indicated restricted gene flow between both *P. infestans* populations from potato (VALLEY and RURAL) and wild *Solanum* hosts (WSS) (Table 7). On average, less than one migrant per sexual generation was estimated between potato and *S. demissum* and *S. xedinese* populations (Table 7). Low differentiation was measured between VALLEY and RURAL *P. infestans* populations (Table 7). Gene flow was generally greater between VALLEY and RURAL populations compared with the WSS population when fine-grain comparisons between pairs of subpopulations were made (Table 7). Significant population subdivision was detected within the WSS population. Patches 3 and 4 showed a significant differentiation ($P = 0.001$) when using the exact test for population differentiation. Most of the population substructuring could be attributed to differences at the population level, whereas only certain WSS patches differentiated at the subpopulation level (Tables 6 and 7; Fig. 4).

TABLE 5. Pairwise comparisons of population differentiation in populations of *Phytophthora infestans*^a

Population	VALLEY	RURAL	WSS
VALLEY	...	4.417	1.053
RURAL	1.000	...	0.785
WSS	0.001	0.022	...

^a Below the diagonal, probabilities for each pair-wise comparison using an exact test (36). Above diagonal, estimates of pair-wise number of migrants (N_m).

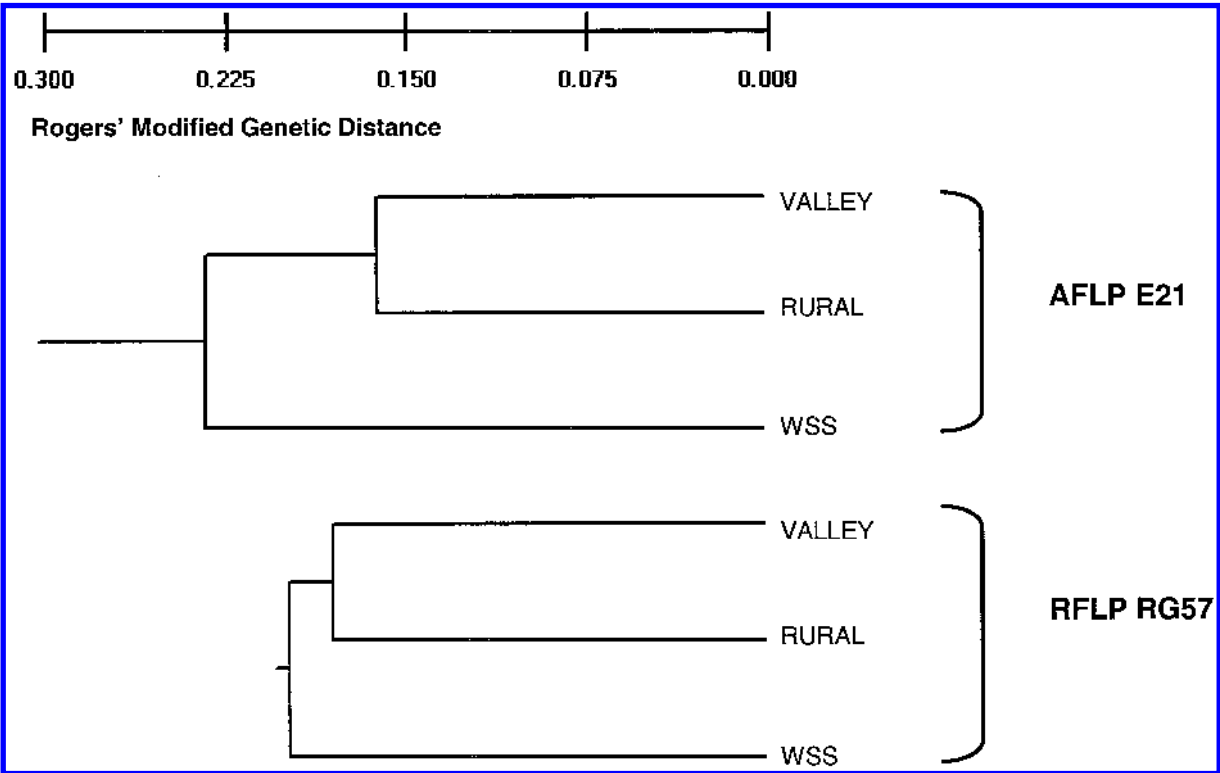


Fig. 2. Comparison of genetic similarity of three subpopulations of *Phytophthora infestans* collected within the Toluca Valley using neutral DNA markers generated by either amplified fragment length polymorphism (AFLP) or restriction fragment length polymorphism (RFLP) fingerprinting. Genetic distances were estimated using Rogers' modified genetic distance coefficients (50). Statistical support for the phenogram branches was obtained using 1,000 bootstrapped samples. Dendrogram constructed by unweighted pair-group method with arithmetic average clustering.

DISCUSSION

Isolate diversity. The first goal of our study was to use mtDNA and AFLP markers to characterize the genetic structure of *P. infestans* populations located in the Toluca Valley, central Mexico. AFLP markers used in this study revealed a high degree of genetic variability in the three *P. infestans* populations sampled in the Toluca Valley. Almost every single isolate represented a unique genotype based on 165 dominant AFLP markers. Our observations confirm previous reports (20,22,29) of high genetic diversity of *P. infestans* in central Mexico and add to the extensive evidence that support the hypothesis of central Mexico being the center of diversity and probably the center of origin of the late blight pathogen (14,20). No variation was found for mtDNA markers because only the Ia haplotype was detected. Our results confirm that haplotype Ia still represents the predominant mtDNA haplotype in *P. infestans* populations in the highlands of central Mexico. Together with haplotypes IIa and IIb (7,21), Ia has been found among isolates, associated with worldwide migration during the 1970s (13). The tight association between haplotype Ia and variable, often sexually reproducing *P. infestans* populations, provides strong circumstantial evidence that Ia rather than haplotype Ib represents the ancestral type of mitochondrial haplotype in *P. infestans* (37). Intermediately metalaxyl resistant or resistant isolates were detected in all three populations. Metalaxyl resistance was fairly common among isolates collected from *S. demissum* (22,29) and was also detected in *P. mirabilis* isolates growing along roadsides and in natural vegetation in the Toluca Valley in 1998 and 1999 (W. G. Flier and N. J. Grünwald, unpublished data). The question may be raised whether Ridomil would have been introduced as a fungicide against *P. infestans* if the baseline

monitoring of metalaxyl had been performed using a population of the pathogen with a broad genetic variation as is present in the Toluca Valley.

Differentiation of *P. infestans* populations. We tested the hypothesis that populations of *P. infestans* on native *Solanum* spp. are merely derived from populations on cultivated potatoes. This hypothesis implies a decrease in genotypic diversity in the WSS population. We did not find evidence for reduced genetic diversity among *P. infestans* strains collected from native *Solanum* spp. based on AFLP marker data. Instead, subpopulations of *P. infestans* on wild *Solanum* hosts were among the most variable populations found in the Toluca Valley. Our results are therefore not consistent with the earlier report (22), which suggested that WSS populations are less diverse than RURAL or VALLEY populations based on mating type and *Pep* and *Gpi* allozyme markers. Insensitivity to metalaxyl proved not to be a very informative marker for gene flow studies in *P. infestans* populations from the Toluca Valley. Metalaxyl insensitive isolates were collected from all three populations and relatively high numbers of intermediate or resistant isolates were detected in *P. infestans* collections from *S. xedinense*. The slightly higher frequency of metalaxyl resistant strains found on *S. xedinense* compared with collections from *S. demissum* might be explained by the difference in ecological habitats between the two native host species. *S. xedinense* is commonly found in rural, low-input potato fields in which they mingle with the Criolla potato crops. In contrast, *S. demissum* is usually found under pine trees in natural habitats. It is therefore very likely that *P. infestans* isolates from *S. xedinense* have had a higher chance of repeated exposure to metalaxyl compared with isolates from *S. demissum*. It has been shown (9) that repeated exposure to metalaxyl will soon lead to the occurrence of

TABLE 6. Matrix of genetic similarity and distance coefficients for *Phytophthora infestans* populations and subpopulations^a

Population	Subpopulation	Field station	Commercial fields	Criolla's	Patch 1	Patch 3	Patch 4
VALLEY	Field station	...	0.985	0.991	0.957	0.959	0.950
VALLEY	Commercial fields	0.129	...	0.983	0.970	0.976	0.961
RURAL	Criolla's	0.105	0.129	...	0.961	0.957	0.948
WSS	Patch 1	0.204	0.168	0.192	...	0.976	0.952
WSS	Patch 3	0.201	0.152	0.198	0.153	...	0.947
WSS	Patch 4	0.220	0.190	0.219	0.212	0.222	...

^a Below the diagonal, Rogers' modified genetic distance; above the diagonal, Nei's genetic identity.

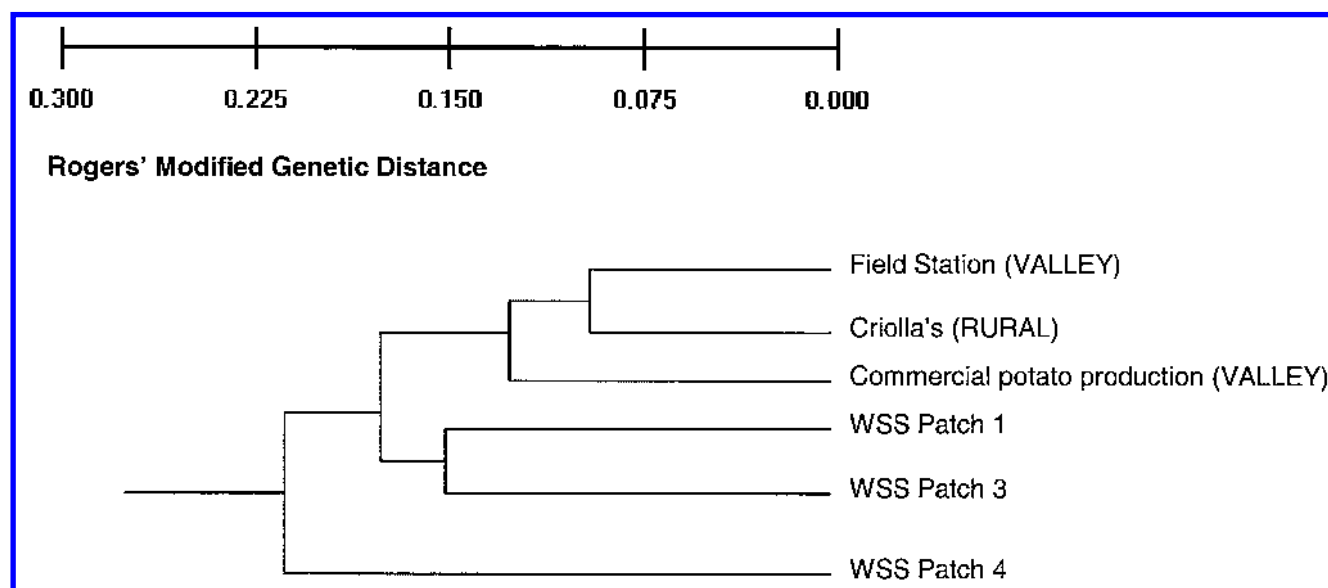


Fig. 3. Cluster analysis of six subpopulations of *Phytophthora infestans* in Toluca Valley using 165 putative amplified fragment length polymorphism loci. Distance was estimated using Rogers' modified genetic distance coefficients (50). Dendrogram constructed by unweighted pair-group method with arithmetic average clustering.

phenylamide resistance in field populations of *P. infestans*. The presence of metalaxyl resistant isolates on native *Solanum* spp. has been repeatedly used to argue against the presence of genetic differentiation between *P. infestans* from potato and native *Solanum* hosts (14,29). The hypothesis of panmixis can however not explain the presence of considerable levels of metalaxyl resistance in *P. mirabilis* (8.7% of *P. mirabilis* isolates being intermediate or resistant, $n = 69$). Similar observations have been made when studying *P. infestans* populations from Rio Negro, Colombia, and Rwanda where widespread resistance against metalaxyl was found present well before the introduction of phenylamide-based fungicides (1985 to 1989) (L. J. Turkensteen, unpublished data).

Based on neutral marker data, Goodwin et al. (19) concluded that *P. infestans* and *P. mirabilis* should be regarded as separate species. Gene flow was estimated at an Nm value of 0.38, indicating highly restricted or no genetic exchange between the two species. The use of metalaxyl resistance as an indirect marker for gene flow between *P. infestans* populations on different host plant species is therefore ambiguous. Our suggestion in an earlier report (22), stating that gene flow between VALLEY, RURAL, and WSS populations of *P. infestans* based on metalaxyl resistance data is common, should be reconsidered. The presence of a relatively large number of private AFLP alleles in the WSS population suggests that gene flow between *P. infestans* populations on native *Solanum* spp. and cultivated potatoes has been restricted. The exact test for population differentiation based on multilocus genotypes and indirect estimation of gene flow between populations using Nm as a measurement of population differentiation supports this view. On average, migration was less than one migrant per oospore generation between *P. infestans* populations from potatoes and *S. demissum* or *S. xedinense* populations. No restrictions in gene flow were measured between *P. infestans* populations from

cultivated potatoes (VALLEY and RURAL). The measurements of population differentiation combined with the observed Nm values suggest that the *P. infestans* population on native *Solanum* spp. tend to evolve independent from the populations that can be found on cultivated potatoes, which could eventually lead to sympatric speciation (6).

We considered two alternative hypotheses to explain the existing genetic differentiation between *P. infestans* strains from potato crops and native *Solanum* hosts. One hypothesis is that genetic differentiation between *P. infestans* populations can be attributed to spatial and temporal features of late blight epidemics. This cause of genetic subdivision develops only when gene flow is restricted and populations are separated for long periods of time (2). None of these two limiting requirements are in concordance with our current knowledge of potato production in the Toluca Valley. During the 1950s, commercial potato production was intentionally introduced to the Toluca Valley (33). Geographical isolation between native *Solanum* spp. and cultivated potatoes is very limited, and lies often in the order of magnitude of 0.1 to a few kilometers. Late blight epidemics within the valley usually start at the beginning of July (22,24) in the central valley, although we have never observed epidemics of late blight on native *Solanum* spp. before the end of August or beginning of September. The delayed onset of late blight epidemics on native *Solanum* spp. suggest a temporal effect and support the hypothesis that WSS populations are derived from VALLEY or RURAL populations, which is obviously not supported by our AFLP marker data. We conclude that it is therefore not sufficient to explain the observed differentiation between *P. infestans* populations based on spatial and temporal effects. A second hypothesis to explain substructuring is that genetic isolation mechanisms prevent panmixis between populations of *P. infestans* from the Toluca Valley. Host-

TABLE 7. Probabilities of pair-wise population differentiation and migration in *Phytophthora infestans*^a

Population	Subpopulation	Field station	Commercial fields	Criolla's	Patch 1	Patch 3	Patch 4
VALLEY	Field station	...	4.050	5.069	2.003	1.440	2.162
VALLEY	Commercial fields	1.000	...	13.261	3.821	3.403	2.387
RURAL	Criolla's	1.000	1.000	...	3.626	2.667	2.647
WSS	Patch 1	1.000	0.996	0.999	...	5.250	2.461
WSS	Patch 3	0.999	0.944	0.464	1.000	...	1.614
WSS	Patch 4	1.000	0.366	0.210	0.958	0.001	...

^a Below the diagonal, probabilities for each pair-wise comparison using an exact test (36); above the diagonal, estimates of pair-wise number of migrants (Nm).

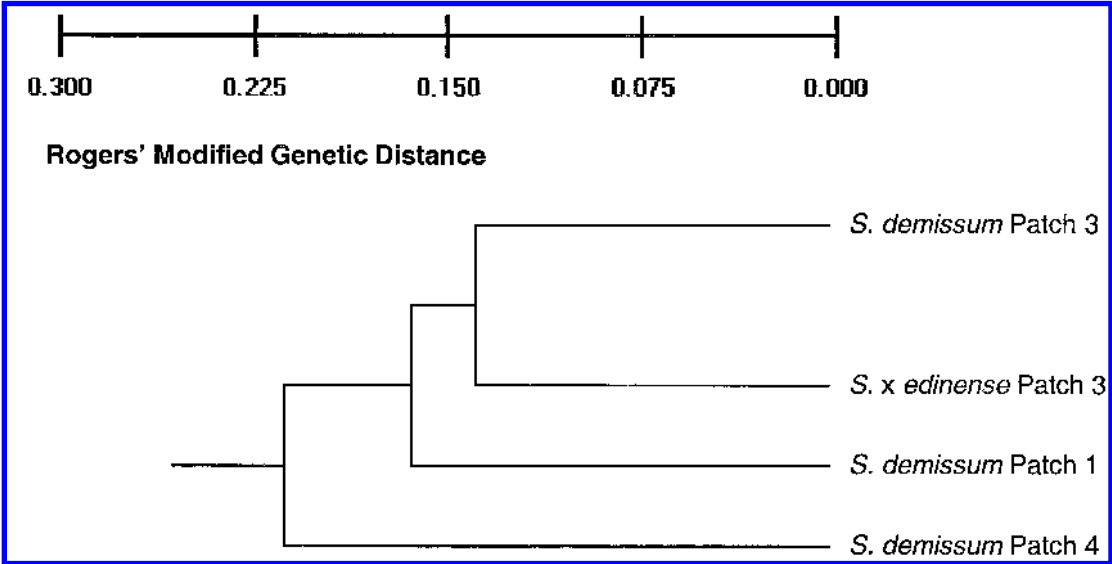


Fig. 4. Cluster analysis of four populations of *Phytophthora infestans* collected from patches of *Solanum demissum* or *S. xedinense* from the Toluca Valley using 165 putative amplified fragment length polymorphism loci. Distance was estimated using Rogers' modified genetic distance coefficients (50). Dendrogram constructed by unweighted pair-group method with arithmetic average clustering.

specific compatibility genes involved in host specificity as well as reproductive barriers might contribute to this type of isolation. At least 11 race-specific virulence genes (R-genes) have been identified in native *Solanum* spp. from the central highlands of Mexico (4,28), and it is generally believed that these 11 R-genes represent a small proportion of the R-genes present in this area. The presence of specific resistance genes (and the numerous combinations of R-genes in individual *Solanum* genotypes) could serve as an explanation for the observed reduction in gene flow between *P. infestans* from cultivated potatoes and native *Solanum* spp. Additional evidence supporting the view that R-genes and host specificity contribute to population differentiation is presented by Ordoñez et al. (35). They reported on an Ecuadorian *Phytophthora* A2 population closely resembling *P. infestans* that appears to be strictly isolated from potato strains by host-plant specificity. In addition, significant differences in oospore production were observed between in vitro pairing of isolates collected from potato and *S. demissum* (12). Isolates originating from the native host *S. demissum* produced significantly more oospores in crosses with compatible strains collected from the same host species compared with crosses with isolates collected from cultivated potatoes. Isolation mechanisms based on host specificity and reproductive success requires strategies that facilitate the survival of the pathogen on alternative hosts during the dry season when foliage of both potato crops and wild hosts is not present. Extensive monitoring of late blight epidemics on wild tuber-bearing *Solanum* spp. in the Toluca Valley during the 1980s led to the conclusion that hibernation of mycelium in infected tubers of native *Solanum* spp. cannot be regarded as a common source of initial inoculum for *P. infestans* (39). There is however strong evidence that oospores produced in leaflets of *S. demissum* with multiple late blight lesions contribute to the survival during the host-free period (12).

Ongoing sympatric speciation in *P. infestans*? Ordoñez et al. (35) provided evidence suggesting that sympatric speciation, the origin of two or more new species from a single local ancestral population without geographical isolation (15,25,30), may be possible in *P. infestans*. Restricted gene flow and reported differences in reproductive success between *P. infestans* populations from native *Solanum* spp. and cultivated potatoes support the hypothesis of population differentiation in local populations of *P. infestans* in the Toluca Valley of central Mexico. It has been stated that population differentiation and sympatric speciation can be accelerated by either resource use through host adaptation (strong selection for virulence genes in the pathogen to neutralize R-genes in *S. demissum*) or random genetic drift (3,10,30). It is likely that *P. infestans* populations infecting patches of *S. demissum* undergo severe bottlenecks at the end of each growing season, so it is expected that random genetic drift plays an important role in shaping populations of *P. infestans* that are very restricted in size. The presence of recurrent population bottlenecks at the end of the growing season has a pronounced effect on the differentiation for neutral genes and the time to reach equilibrium (2). Our study provides evidence suggesting the presence of population differentiation in local populations of *P. infestans*. High genetic identities and fairly high gene flow estimates among the three pathogen populations indicate that host specialization and temporal effects do not completely prevent migration of *P. infestans* strains between solanaceous hosts in the Toluca Valley. We hypothesize that the genetic architecture of *P. infestans* in the Toluca Valley is driven by host-specific factors (i.e., R-genes) widely distributed in wild *Solanum* spp. and random genetic drift.

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